IL-10 induces IgG4 production in NOD-scid Il2r null mice humanized by engraftment of peripheral blood mononuclear cells

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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IL-10 induces IgG4 production in NOD-*scid* Il2rγ^{null} mice humanized by engraftment of peripheral blood mononuclear cells

To the Editor,

IgG4 antibodies are considered to have anti-inflammatory activity and may confer protection against anaphylaxis and allergic inflammation. Strong increases in the production of allergen-specific IgG4 antibodies are frequently observed in allergic patients receiving allergen-specific immunotherapy (AIT).[1] The production of IgG4 by human B cells *in vitro* is strongly enhanced by IL-10.[2,3] *In vivo* studies of the regulation of IgG4 have been handicapped by the fact that mice do not express this immunoglobulin isotype. As a result, conventional mouse models and gene-deficient or transgenic mice cannot be used to study aspects of the regulation of IgG4. The use of humanized mice allows the study of human immunoglobulin regulation including IgG4. Immunodeficient NOD.*cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (alternatively named NOD-*scid Il2rγ*^{null} or NSG) mice lack murine lymphocytes, including T, B, and NK cells. This strain enables efficient engraftment of human hematopoietic progenitor cells (HPC) and peripheral blood mononuclear cells (PBMC).[4] While human lymphocytes engraft relatively well in NSG mice, there are certain limitations to this model, such as the low levels of human cytokines and growth factors present in serum and tissues, HLA incompatibility between engrafted lymphocytes and murine host cells, which will eventually culminate in graft-versus-host disease. Moreover, NSG mice have underdeveloped secondary lymphoid tissues. While engrafted human T and B cells populated lymph nodes and spleen, these tissues will not fully resemble human secondary lymphoid tissues.[5] Nevertheless, this model has been successfully used to study allergen-specific T-cell responses.[6] Here, we established a humanized mouse model to optimize B-cell and plasma cell engraftment and to study the regulation of human immunoglobulin production *in vivo*.

To determine optimal conditions for B-cell engraftment in NSG mice, engraftment of human leucocytes after intraperitoneal (IP) and intravenous (IV) injections of 5x10^6 and 20x10^6 PBMC was analyzed after 14 and 21 days in spleen. Engraftment of human cells was determined by flow cytometry. Expression of human (hu)CD45 was used for gating of human leukocytes (Figure). Successful engraftment of human cells was associated with increased spleen size (Figure S1). In control mice that did not receive PBMC, no huCD45+ cells were detected (Figure 1A-D). Engraftment was not efficient (<1% huCD45+ splenocytes) for one out of 4 PBMC donors, and mice injected with these PBMC were excluded from the analysis. The percentages of huCD45+ cells ranged from 20% (in the 5 × 10E^6 IV group) to 70% (in the 20 × 10E^6 IV and 20 × 10E^6 IP group) on day 14 and from 50% (5 × 10E^6 IV group) to 90% (in the 20 × 10E^6 IV group) on day 21 (Figure 1B). The highest level of engraftment of huCD45+ cells was observed 21 days after IV injection of 20 × 10^6 PBMC. No significant differences were found between the IV and IP groups in terms of total splenocyte counts and absolute numbers of huCD45+ cells (Figure 1B). Human B lineage cells (gated as huCD45+CD19+) were detectable at low levels on day 14 in the IV group and in both the IV and IP group on day 21 (Figure 1E,F). To differentiate between B cells and plasma cells, we gated B cells as CD19+CD138- (Figure 1E,G) and plasma cells as CD138+ (Figure 1E,H). Total CD19+ B lineage cells, CD19+CD138- B cells, and CD138+ plasma cells showed a similar pattern in which the highest level of engraftment, both as a percentage of huCD45+ cells and in absolute engrafted cell numbers, was observed with IV injection of 5 × 10^6 PBMC. The higher B lineage engraftment observed in the 5 × 10^6 IV group coincided with reduced T-cell frequencies (Figure 1I,J, Supplementary Figure 2A). Interestingly, the IV route of application was associated with a trend toward higher frequencies of CD4+ T-cell engraftment, while the IP route was associated with increased CD8+ T-cell frequencies (Figure 1J-L and Supplementary Figure 2B,C). The small fraction of CD3+CD4-CD8- cells (Figure 1I) may represent natural killer T cells

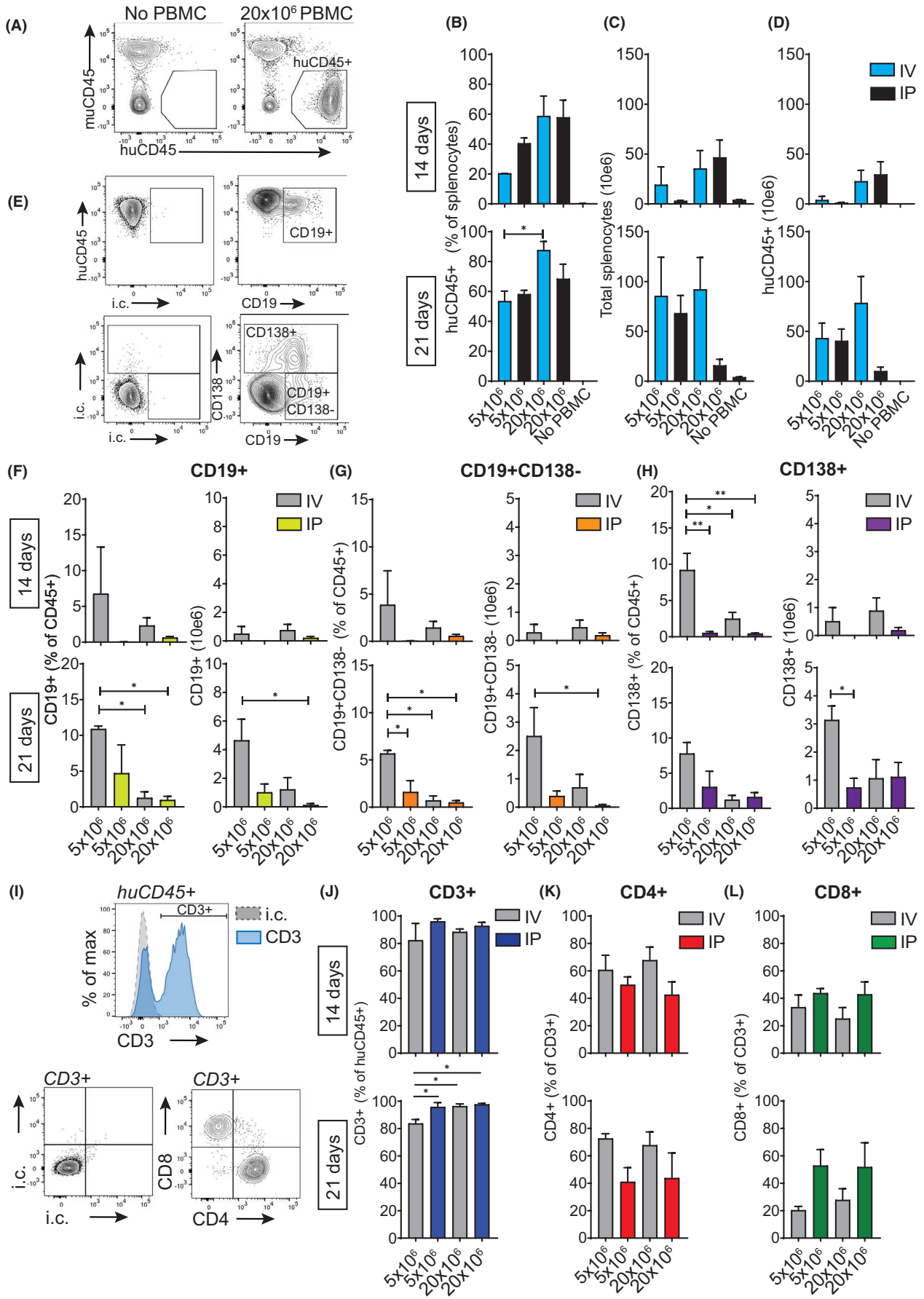
(A)
No PBMC
20x10⁶ PBMC
muCD45
huCD45
huCD45+
(B)
(C)
(D)
14 days
21 days
huCD45+ (% of splenocytes)
Total splenocytes (10e6)
huCD45+ (10e6)
IV
IP
5x10⁶
5x10⁶
20x10⁶
20x10⁶
No PBMC
(E)
huCD45
i.c.
CD19+
CD19
CD138+
CD138
CD19+
CD138-
(F)
CD19+
(G)
CD19+CD138-
(H)
CD138+
CD19+ (% of CD45+)
CD19+ (10e6)
CD19+CD138- (% of CD45+)
CD19+CD138- (10e6)
CD138+ (% of CD45+)
CD138+ (10e6)
(I)
huCD45+
CD3+
i.c.
CD3
% of max
CD3
CD3+
CD3+
i.c.
i.c.
CD8
CD4
(J)
CD3+
(K)
CD4+
(L)
CD8+
CD3+ (% of huCD45+)
CD4+ (% of CD3+)
CD8+ (% of CD3+)

FIGURE 1 Optimization of human lymphocyte engraftment in NSG mice. (A) Representative dot plots showing muCD45 and huCD45 staining in splenocytes of a mouse that did not receive PBMC and a mouse that was engrafted with human PBMCs. (B) Frequencies of huCD45+ cells in splenocytes of NSG mice 14 and 21 days after injection of 5×10^6 or 20×10^6 PBMC through IV or IP injection. (C) Total cell counts per spleen. (D) Total number of engrafted huCD45+ cells. (E) Left: Isotype control (i.c.) for CD19 and CD138, right: gating strategy for CD19+, CD19+CD138- and CD138+ cells. Dot plots show huCD45+ splenocytes. (F) Frequencies (left) and absolute numbers per spleen (right) of CD19+, (G) CD19+CD138−, (H) CD138+ cells. (I) Dotted line in histogram: isotype control (i.c.) for CD3. Bottom left dot plot: i.c. for CD4 and CD8. Bottom right: gating strategy for CD4+ and CD8+ T cells. Frequencies of (J) total CD3+ T cells, (K) CD4+ and (L) CD8+ T cells. $^*p < 0.05$, $^{**}p < 0.01$. Statistics were calculated using one-way ANOVA followed by Tukey's multiple comparison test. Graphs show mean ± SEM from three mice per group

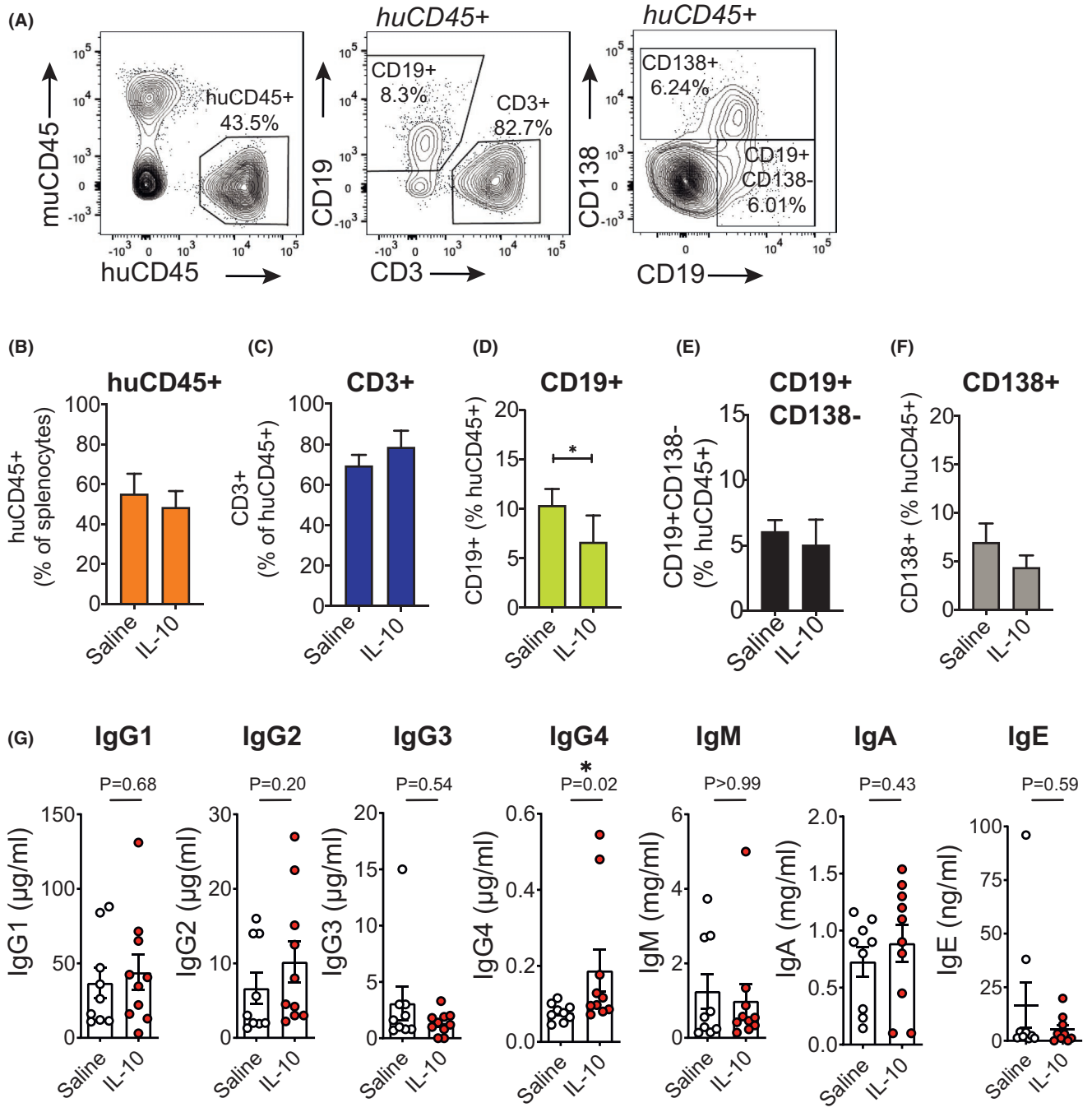

FIGURE 2 Administration of IL-10 to humanized NSG mice results in elevated IgG4 production *in vivo*. (A) Gating strategy for gating of huCD45+, CD3+, CD19+,CD19+CD138−, and CD138+ cells. (B-F) Frequencies at 21 days after PBMC engraftment in spleens of human huCD45+ (B), CD3+ T cells (C), CD19+ B lineage cells (D), CD19+CD138- B cells (E), and CD19+CD138+ plasma cells (F). (G) Serum immunoglobulin levels measured 21 days after PBMC engraftment in mice treated with saline or IL-10. Statistics were calculated using Mann-Whitney test, $^*p < 0.05$. Graphs show mean ± SEM from 9 mice (saline-treated) and 10 mice (IL-10 treated)

or γδ-T cells. We concluded that the IV route with $5x10^6$ PBMC was optimal for B lineage and CD4+ T helper cell engraftment.

Next, we assessed the effect of IL-10 on the B-cell compartment and immunoglobulin production. IP injection of 3 μg IL-10 at day 3 and day 12 after PBMC engraftment did not significantly affect the engraftment of huCD45+ cells or CD3+ T cells (Figure 2A-C). The frequencies of CD19+ cells were significantly reduced in the IL-10-treated group (Figure 2D), while CD19+CD138- B cells (Figure 2E) and CD138+ plasma cell (Figure 2F) frequencies showed trends of reduction, with no statistically significant difference. All human immunoglobulin isotypes were detectable in serum of IL-10-treated or saline-treated control mice (Figure 2G). Interestingly, IL-10 induced a significant increase in the serum level of IgG4, while no significant changes were observed for IgG1, 2, 3, IgM, IgA, and IgE. Macrophages are involved in the regulation of acquired immune responses in the spleen. Because IL-10 is a known inhibitor of macrophage activation, it could be speculated that IL-10 may affect different features of macrophages.

These data show that NSG mice can be used to efficiently engraft human B cells and plasma cells. However, it should be emphasized that this model has several limitations. Firstly, although human immunoglobulin isotypes are readily detectable in humanized NSG mice, murine Fcγ receptors have different affinities for human IgG subclasses than their human counterparts. Therefore, an increase in IgG4 might not be functional in future studies due to human aberrant or abrogated Fc fragment-murine Fc receptor interactions. We observed a relatively large degree of variation in human immunoglobulin levels between individual mice within the same treatment groups (Figure 2G). This may result from differences in the degree of plasma cell differentiation and survival or class switch recombination between individual mice. Such variations may impair the detection of subtle effects in more advanced studies involving antigen-specific responses. Moreover, it remains to be determined whether allergen-specific B-cell and antibody responses can be efficiently induced in this model. Taken together, our findings demonstrate that human PBMC-engrafted NSG mice can be utilized to study human B-cell and immunoglobulin responses. $5X10^6$ PBMC and application through IV injection were optimal for B-cell and plasma cell engraftment and IL-10 treatment stimulated the *in vivo* upregulation of IgG4 production. In the allergy field, this model may be further developed to study *in vivo* antigen-specific B-cell and antibody responses. It may be used to test novel AIT approaches, and combining this model with transfer of allergen-specific T-cell lines (as reported by Vizzardelli et al.)[6] will allow in depth analysis on the effects of different T-cell subsets on B-cell and antibody responses.

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CONFLICT OF INTEREST

The authors declare that they have no relevant conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Clinical and genetic features of hereditary angioedema with and without C1-inhibitor (C1-INH) deficiency in Japan

To the Editor,

Hereditary angioedema with normal C1-inhibitor (HAEnCI) is an umbrella term for several types of HAE that phenotypically resemble HAE but in which variants affecting function have been identified in genes other than the C1-INH gene (*SERPING1*).[1] In contrast to European patients with HAE, we suppose comprehensive clinical and genetic data of HAE are scarcely reported from Asian countries, including Japan. In particular, HAEnCI in Asia has not been clearly characterized. Considering ethnic differences, it would be important to clarify the features of HAE in the Asian population. Here, we report the clinical and genetic features of Japanese patients with 158 cases from 122 families with HAE-C1-INH and 21 cases from 21 families with HAEnCI. HAEnCI was defined as follows: normal C1-INH activity, no variants affecting function in the *SERPING1* gene, at least one relative with recurrent angioedema attacks (i.e., positive family history), no history of urticaria, and lack of efficacy by antihistamines or corticosteroids.

Table 1 shows clinical features of our Japanese patients with HAE-C1-INH and HAEnCI. When compared with HAE-C1-INH, HAEnCI was significantly more predominant among females (95.2% vs. 66.9%, $p = .008$), more frequently affected in the face (61.9% vs. 32.9%, $p = .009$) and pharynx/larynx (47.6% vs. 21.5%, $p = .009$), associated with more frequent exacerbations in the previous year (mean ± SD: 14.4 ± 27.3 vs. 3.66 ± 7.14, $p = .0001$), associated with higher prevalence of patients with more than six angioedema attacks in the previous year (52.4% vs. 16.5%, $p = .0001$), and more susceptible to triggers such as physical stress (38.1% vs. 12.7%, $p = .007$) and upper respiratory infections (URI) (19.0% vs. 5.1%, $p = .037$). The prevalence of having a positive family history experiencing angioedema attacks was significantly higher in HAEnCI (100%) than in HAE-C1-INH (81.6%) ($p = .027$). This is because the inclusion criteria of HAEnCI demand a family history of angioedema. There were no differences in most of the clinical features of male and female patients with HAE-C1-INH except the incidence of urological attacks and prodromal symptoms (Table S1).

It is of note that our patients with HAEnCI suffer from edema in the face and pharynx/larynx twice more frequently than those with HAE-C1-INH. In European patients with HAEnCI, facial and oropharyngeal swellings develop more predominantly than those with HAE-C1-INH as well.[2] On the other hand, there are a number of differences in the clinical characteristics such as frequency of attacks and triggers between Japanese and European patients with HAEnCI. In particular, hormonal perturbations induced by menstruation/pregnancy or oral contraceptives did not seem to influence attacks in our patients with HAEnCI. This finding is in contrast with European patients with HAEnCI whose angioedema symptoms are frequently deteriorated by oral contraceptives or pregnancies.[2]

In the case of HAE-C1-INH, the incidence of abdominal attacks was different between our patients and European patients. Abdominal attacks are common clinical manifestations of European patients with HAE-C1-INH, whose incidence reaches over 90% in these patients.[3] In contrast, the proportion of patients who had experienced intestinal attack was 35.4% in our 158 patients with HAE-C1-INH (Table 1). Other reports from Asia such as the one from Mainland China[4] have shown similar incidence of abdominal attacks with the present study.

Genetic analysis directed toward the entire exons for the known causative genes was performed for our Japanese HAEnCI patients